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13. SUPPLEMENTARY NOTES

14. ABSTRACT Funds provided by this grant were used to purchase instruments including fluorescence microscopes and an ultra-speed refrigerated centrifuge to establish the Bioengineering, Cell Biology and Tissue Culture laboratory and for student research projects. Undergraduate students and local high school teachers received training in the cell biology laboratory that was established in the Fall 2000. The laboratory was used as a teaching lab for the Cell Biology course offered by the PI, for research projects for pre-med students and students supported by the Alliance for Minority Participation (AMP) program headed by Mario Diaz, Ph.D., Chairman of the Engineering Technology Department. The nascent laboratory is part of an embryonic group for the establishment of a Bioengineering Program at the university. It was impossible however to establish the basic science research program initially envisioned by the PI. Finding adequate space for the laboratory was not realized until June 2000, furnishing the laboratory with discarded workbench obtained from the university warehouse and disinfecting all the cabinets and drawers was not accomplished until mid-August 2000, the ultra-speed centrifuge was not received until late fall 2000 due to the cumbersome purchasing process, and most significantly, the matching funds promised at the time of request for this grant were not made available for use by the PI. We did however have several successes: 1. for the first time in UTB history, the Cell Biology students had access to a functional laboratory and conducted real experiments independently; 2. three students with diverse backgrounds worked together in a study of the chloroplast and red blood cell membranes using the atomic force microscope and the microscopes purchased with these funds; 3. several pre-med students conducted short, independent research projects in the laboratory; 4. the laboratory was used to conduct two in-service sessions on Biotechnology for Brownsville Independent School District science teachers; and 5. the laboratory was used to conduct a two-week short course on Environmental Toxicology for eight students and one professor from the InterAmerican University of Puerto Rico, Barranquitas, P.R. The goal of establishing a functional Cell Biology laboratory is almost attained.

15. SUBJECT TERMS

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FINAL REPORT

GRANT NUMBER: F49620-99-1-0327

FY99 DoD HBCU/MI Infrastructure Support Program
(Instrumentation)

Title of Project: The Use of Fluorescent-labeling Compounds in Demonstrating Gene Delivery and Cytoplasmic Translocation

Principal Investigator: Juan G. Guevara, Jr., Ph.D.
Associate Professor, Department of Biological Sciences
The University of Texas @ Brownsville
80 Fort Brown
Brownsville, Texas 78520

Funds provided by this grant were used to purchase instruments including fluorescence microscopes and an ultra-speed refrigerated centrifuge to establish the Cell Biology laboratory and for student research projects. Undergraduate students and local high school teachers received training in the cell biology laboratory that was established in the Fall 2000. The laboratory was used as a teaching lab for the Cell Biology course offered by the PI, for research projects for pre-med students and students supported by the Alliance for Minority Participation (AMP) program headed by Mario Diaz, Ph.D., Chairman of the Engineering Technology Department. The nascent laboratory is part of an embryonic group for the establishment of a BioEngineering Program at the university. It was impossible however to establish the basic science research program initially envisioned by the PI. Finding adequate space for the laboratory was delayed by almost two years and processing purchase requests was cumbersome and also resulted in a long delay for setup. Several successes can be listed: 1. Cell Biology students had access to a functional laboratory and conducted real experiments with minimal supervision; 2. A new BioEngineering Program in the Department of Physics is being established and will have use of the laboratory; 3. Pre-Med students will have a laboratory available for their individual biomedical research projects; and 4. Several students for research projects including one project using the Atomic Force Microscope have used the laboratory.

Introduction: The instruments purchased with the ARO funds are now part of a well-equipped Cell Biology laboratory that has been used in the last twelve months for teaching undergraduate students methods in cell biology research and for introducing local high school teachers to biotechnology. Further, several undergraduate students and one high school chemistry teacher have participate in entry level research projects ranging from the use of the atomic force microscope to the use of an ultra-speed centrifuge for isolation of low-density lipoproteins. Undergraduate research projects were conducted in the nascent laboratory using these instruments. The PI conducted two in-service workshops for the Brownsville Independent School District science teachers in the laboratory. A group of

students and one professor from the InterAmerican University of Puerto Rico also benefited from our new laboratory. This group came for a short course in Environmental Toxicology that was presented by the PI and funded by the USDA. The instruments were described and a brief lecture on their utilization in basic and applied research was presented.

Accomplishments related to this grant:

1. The BioEngineering, Cell Biology and Tissue Culture Laboratory was established in June 2000 and is located in the Science, Engineering and Technology Building (SETB) in room Q1.414 (~800sq.ft.). It is used as a teaching and research lab space. The Beckman Optima LE-80K Preparative Ultra-centrifugation with rotors purchased with AFOSR grant funds is located in Q1.414 and will be used in the isolation and purification of lipoproteins and cellular organelles. In addition to the ultra-centrifuge, other instruments are located in this laboratory including a thermal cycler, micro plate reader, spectrophotometer, P-2 level laminar flow biosafety hood, top-loading and analytical balances, a Silicon Graphics, Inc. O2 Molecular Modeling Workstation with INSIGHT II software, one tissue culture CO_2 incubator, several electrophoresis apparatuses with power supply units, one refrigerator, one REVCO –80C freezer, one hybridization oven, a micro centrifuge, a refrigerated table-top low-speed centrifuge, two ultra-violet light boxes, three digital cameras, three pc's, and a Barnstead Nanopure Water-filtration System.

This laboratory has been used by students enrolled in the Cell Biology course as a their laboratory. In this course, students were taught how to organize a research project, conduct the necessary library research to establish experimental procedure and protocols, and perform experiments in the laboratory. Research students have learned to isolate chloroplasts from native and non-native woody legumes, to isolate the chloroplast chromosome, and to isolate lipoproteins and macrophages.

The Molecular Modeling Workstation has been used by students to learn about structure and functional relationships in macromolecules including enzymatic protein-substrate/product interactions, protein-protein interactions, and protein-nucleic acid binding interactions. Students also get to visualize and study protein secondary structures in 3-D and learn about amphipathic helices. Students have studied class I ATP-binding motifs and compared the structure to a like motif that binds RNA in the KH domain. One student has used molecular modeling to studying the 3-D structure of viral DNA-binding proteins.

2. The Microscopy Laboratory was also established in late summer 2000 and it is located in SETB Q1.408A (~280sq.ft.). A Zeiss AXIOVERT 25 CFL Inverted Microscope for fluorescence and phase contrast and a Zeiss AXIOSKOP 2 MOT Research Microscope for transmitted light bright field, phase contrast and fluorescence with Plan Neofluar Objectives and equipped with an AXIOCAM Ultra high resolution digital color camera were purchased with AFOSR grant funds and are the mainstay instruments in our microscopy laboratory. In addition, there are two Zeiss Inverted microscopes (a refractive light and a bright field) plus two Zeiss microscopes (a refractive light and a bright field). Students used this laboratory to study chloroplasts isolated from various woody legumes and grasses obtained from our campus.

Although almost equipped these laboratories are yet not 100% functional. We are still in need of a chromatography cold chamber, fraction collectors and peristaltic pumps. The instruments including fluorescent compound microscopes, inverted fluorescent microscopes, spectrophotometers and ultracentrifuge that were purchased with ARO funds, have been used in teaching undergraduate students methods and techniques used in cell biology research.

3. A collaborative association between the faculty in the science and technology departments at UTB/TSC and the Brownsville Independent School District was established and has been an effective method of recruiting students to UTB. In the Fall 2001 semester, the PI and Mr. Julio Sada, Chemistry teacher at Rivera High School, established a basic research program for his students who will develop skills in electrophoresis and molecular modeling. These students show interest in science and will perhaps decide to continue their education at UTB or other institutes of higher learning. The immediate aim is to help them develop projects for this year's annual science fair.

4. The major objective of this infrastructure grant was to provide instruments and equipment for the Cell Biology and Immunology laboratories. This was accomplished. In addition, we have been able to establish a program toward a degree in BioEngineering for students who elect Physics as their major (see below). Although our Molecular Cell Biology and Molecular Immunology courses include the study of basic cellular processes (such as signal transduction, activation of transcription), molecular biology, immunology, and gene transfection, the PI prepares the students for these rigorous courses by teaching them how to read the new information, how to become familiar with the new jargon and new concepts as well as how to prepare for the essay examinations given in the course. One aim in the Cell Biology course has been to prepare students with a strong foundation in cell biology and in the scientific investigative process for careers in the growing medically-related and biotechnological industries. Students were taught how to plan and conduct experiments; how to collect, analyze and interpret data; how to keep accurate records; and how to present their results to others through poster presentations, slide presentations, or written reports. Students were expected to read current science articles in support of topics covered in lectures. Furthermore, the PI established a journal club for students interested in honing their verbal presentation skills. Also, MCAT study sessions were established for students in pre-med programs. The PI prepared a degree plan for pre-med students to aid them in preparing for the MCAT examination. Unfortunately, the lack of additional funds needed to obtain a high performance liquid chromatography system, a chromatography cold chamber, and supplies for the cell culture experiments made it impossible to conduct the cell studies described in the original grant application.

5. The following degree plan was suggested to the Department of Biology for preparing our students for the MCAT.

COURSES REQUIRED BY U.T. SYSTEM MEDICAL SCHOOLS

General Biology (lecture 1306, lab 1106)
General Biology (lecture 1307, lab 1107)
General Chemistry I (lecture 1311, lab 1111)
General Chemistry II (lecture 1312, lab 1112)
Organic Chemistry I (lecture , lab)
Organic Chemistry II (lecture , lab)
General Physics I (lecture 1301, lab 1101)
General Physics II (lecture 1302, lab 1102)
Mathematics, Calculus I

COURSES RECOMMENDED IN PREPARATION FOR MCAT

General Microbiology
Cell Biology
Genetics
Advanced Physiology
Immunology
Molecular Biology
Anatomy
Physics of Biological Systems
Analytical Chemistry
Physical Chemistry
Biochemistry
Biomedical Research Seminar

RECOMMENDED PRE-MED/PRE-DENT DEGREE PROGRAM

Course Title	Semester Hours	Total Hours
Required Courses	40	40
Principles of Biology I & II (with labs)	8	
General Chemistry I & II (with labs)	8	
Organic Chemistry	8	

I & II (with labs)		
General Physics I & II (with labs)	8	
Calculus I	3	
		35
Electives		
General Microbiology (with lab)	4	
Cell Biology (with lab)	4	
Genetics (with lab)	4	
Advanced Physiology (with lab)	4	
Immunology (with lab)	4	
Molecular Biology (with lab)	4	
Vertebrate Anatomy (with lab)	4	
Physics of Biological Systems	3	
Analytical Chemistry	3	
Physical Chemistry	6	
Biochemistry (with lab)	8	
		48
	Total	123

WORDS TO THE WISE PRE-MED STUDENT:

Ninety-five percent of the responsibility for learning is yours. We, the faculty, do not take the MCAT with you or score it after you take it. It is our job to help you prepare for this exam by providing you with a rigorous academic program.

DO NOT structure your degree plan as if you are already in medical school, i.e. to address the curriculum program at medical school. Structure your degree plan to prepare you for the MCAT. It is the biggest hurdle you have before entering medical school.

You are not required to major in Biology or Biochemistry. You are required to take the courses listed above.

Development of verbal reasoning, critical thinking and problem solving skills is essential in preparing for the MCAT. You should be reading at least 300 words per minute and understanding 95%.

You must participate in a long-term research project. Identify a research mentor early in your freshman or sophomore years.

You should participate in clubs and societies related to medicine and/or medical research. Dedicate some of your time to community health.

SUGGESTED WEEKLY READING LIST:

Newsweek, Time, and U. S. News and World Report. These are at the high school level.
Scientific American, at college level.
Science, Nature, and Proceedings of the National Academy of Science USA (PNAS, USA).
Journal of the American Medical Association (JAMA) and New England Journal of Medicine.
New Yorker and Harper's
Front pages of Wall Street Journal, New York Times, and Washington Post.

6. **Proposed BioEngineering Program for Physics Department:**

ENGINEERING PHYSICS/BIOENGINEERING

UTB Course Number				
A. Communication (9 hours)	hours	core	Engineering	
ENGL 1301		3	3	3
ENGL 1302 or ENGL 2311		3	3	3
SPCH 1315 or SPCH 1318 (non-ed. Majors)		3	3	3
				9
B. American History Requirements (6 hours)				
HIST 1301		3	3	3
HIST 1302		3	3	3
				6
C. Government Requirements (6 hours)				
GOVT 2301		3	3	3
GOVT 2302		3	3	3
				6
D. Social/Behavioral Science (3 hours)				
Select from:	3 each			
SOCI 2319				
ANTH 2351				
ECON 2301			3	3
SOCI 1301				
PSYC 2301				
GEOG 1303				
				3
E. Kinesiology (1 hour) (health/wellness or activity courses)				
KINE 11XX		1	1	1
KINE 3370 Biomechanics		3		3
				4
F. Literature and Visual and Performing Arts (6 hours)			6	6
ENGL 2322 British Literature I		3		
ENGL 2323 British Literature II		3		
ENGL 2332 World Literature I		3		
ENGL 2333 World Literature II		3		
ARTS 1301 Art Appreciation		3		
ARTS 1303 Art History Survey I		3		
ARTS 1304 Art History Survey II		3		
				6
G. Modern Language (6 hours)			6	6
SPAN 1313, 1314 Elementary Spanish I,II	3,3			
SPAN 1373, 1374 Basic Spanish for Bilinguals I,II	3,3			
FREN 1311, 1312	3,3			
GERM 1311, 1312	3,3			
ITAL 1311, 1312	3,3			
				6
H. Math: (3 hours REQUIRED)				
MATH 2313 Calculus I		3	3	3

MATH 2314 Calculus II		3		3	
					6
I. Science (8 hours REQUIRED)					
BIOL 1306 + 1106 Principles of Biology	3,1			3	
BIOL 1307 + 1107 Principles of Biology	3,1			3	
BIOL 3412 Cell Biology		4		3	
BIOTECHNOLOGY/Topics XXXX		3		3	
CHEM 1311+1111 General Chemistry		3,1	4	4	
CHEM 1312+1112 General Chemistry		3,1	4	4	
CHEM 3301 Inorganic Chemistry		3		3	
PHYS 3315 Physics of biological systems		3		3	
			48		46
J. ENGINEERING TOPICS (48 hrs for eng program)					
RBTC 1405 Robotics Fundamentals		4		4	
ENGR 1204 Engineering Graphics I		2		2	
COSC 1418 Programming Structures I		4		4	
ENGR 2301 Statics		3		3	
				4	*Replace w bioinstr lab,
COSC 2318 Programming Structures II		3		3	
PHYS 2325+2125 Engineering Physics I+ Lab		3,1		4	
PHYS 2326+2126 Engineering Physics II+ Lab		3,1		4	
PHYS 3390 Mathematical Methods in Physicsl		3		3	
PHYS Math Methods in Physics II		3		3	
PHYS 3320 Thermodynamics		3		3	
PHYS 4330 Electromagnetic Theory		3		3	
Total Eng 48					40
				40	
TOTAL HRS					132

Molecular modeling of putative functional regions of apoB-100

Recent studies of apo B-100, the major apolipoprotein of low density lipoprotein (LDL) [1,2] have demonstrated that apoB-100 may have functions other than lipid transport. Sequence similarities were identified between apoB-100 and src-homology domains of signal transduction proteins. Six SH3-like, three SH2-like, and one SH1-like region were suggested to be present in apoB-100. Autophosphorylation of ApoB-100 was observed *in vitro.* This does suggest that *in vivo* this protein may function as a kinase in unknown signal transduction pathway. Furthermore, two putative bipartite nuclear localization sequences (NLS), and four DNA-binding sequences were also located. LDL was shown to bind specific DNA sequences and to translocate the labeled DNA to the nucleus in human fibroblast cells. Sequence comparison indicates that apoB-100 may be involved in signaling as a Signal Transducer and Activator of Transcription (STAT) protein.

Molecular modeling of putative functional kinase region of apoB-100 will be performed using the Insight II (Accerlys, Inc) program. To characterize the src-homology regions of apoB-100, 3D structure of SH1 domains will be produced using consecutive amino acid substitution in the sequences of known kinases of *src* family, followed by the free energy minimization. In a similar fashion, NLS and DNA binding motifs 3D structures will be produced using known functional domains of different transcription factors such as STATs and IRFs. Docking experiments will be performed with DNA-binding domains of apoB-100 and human cytomegalovirus DNA [2].

References

1. Guevara J Jr, Walch ET, Epstein HF, Sparrow JT, Gotto AM, Valentinova NV. Evidence that apoB-100 of low-density lipoproteins is a novel Src-related protein kinase. *J Protein Chem* 1995,**14:** 627-631.

2. Guevara JG Jr, Kang D, Moore JP Nucleic acid-binding properties of low-density lipoproteins: LDL as a natural gene vector. *J Protein Chem* 1999, **18:** 845-857